

***Remarks***

The specification has been amended only to direct entry of the Sequence Listing and to provide the SEQ ID No next to the specific sequence.

In accordance with 37 C.F.R. § 1.821(g), this submission includes no new matter.

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above application are the same.

Applicants respectfully submit that this application is now in condition for examination on the merits.

Respectfully submitted,

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**Version with markings to show changes made**

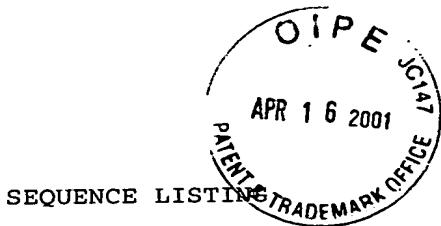
***In the Specification:***

The paragraph bridging pages 93 and 94 has been amended as follows:

Human breast cancer cell lines T-47D and ZR-75-1 were grown according to media component mixtures designated by American Type Culture Collection + 10% FCS (Life Technologies, Inc.), in a 5% CO<sub>2</sub>-95% humidity incubator at 37 °C. T-47D and ZR-75-1 cells were maintained at a cell density between 30 and 80% confluence at a cell density of 0.1 to 0.6 x 10<sup>6</sup> cells/ml. Cells were harvested at 600xg and resuspended at 0.65 x 10<sup>6</sup> cells/ml into appropriate media + 10% FCS. An aliquot of 45 µl of cells was added to a well of a 96-well microtiter plate containing 5 µl of a 10% DMSO in RPMI-1640 media solution containing 0.16 to 10 µM of 2-amino-3-cyano-7-dimethylamino-4-(3-methoxy-4,5-methylenedioxophenyl)-4H-chromene (Example 19) or other test compound (0.016 to 1 µM final). An aliquot of 45 µl of cells was added to a well of a 96-well microtiter plate containing 5 µl of a 10% DMSO in RPMI-1640 media solution without test compound as the control sample. The samples were mixed by agitation and then incubated at 37 °C for 24 h in a 5% CO<sub>2</sub>-95% humidity incubator. After incubation, the samples were removed from the incubator and 50 µl of a solution containing 20 µM of *N*-(Ac-DEVD)-*N'*-ethoxycarbonyl-R110 (SEQ ID NO:1) fluorogenic substrate (Cytovia, Inc.; WO99/18856), 20% sucrose (Sigma), 20 mM DTT (Sigma), 200 mM NaCl (Sigma), 40 mM Na PIPES

buffer pH 7.2 (Sigma), and 500 µg/ml lysolecithin (Calbiochem) was added. The samples were mixed by agitation and incubated at room temperature. Using a fluorescent plate reader (Model 1420 Wallac Instruments), an initial reading ( $T = 0$ ) was made approximately 1- 2 min after addition of the substrate solution, employing excitation at 485 nm and emission at 530 nm, to determine the background fluorescence of the control sample. After the 3 h incubation, the samples were read for fluorescence as above ( $T = 3$  h).

After page 142, the Sequence Listing has been added.



<110> Drewe, John  
Sui Xiong, Cai  
Yan, Wang

<120> Substituted 4H-Chromene and Analogs as Activators of Caspases and Inducers of Apoptosis and the Use Thereof

<130> 1735.0410002/RWE/BEC

<140> 09/705,840  
<141> 2000-11-06

<160> 1

<170> PatentIn version 3.0

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<400> 1

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1